The Relationship between Prodigiosin Biosynthesis and Cyclic Depsipeptides in *Serratia marcescens*

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**Summary**

The concentrations of lipid-bound serine (in the form of cyclic depsipeptide) and prodigiosin, in *Serratia marcescens* respond similarly to changes in environmental or cultural conditions so that highly pigmented cells always have a relatively large amount of lipid-bound serine. In addition, when prodigiosin synthesis is induced in non-pigmented cells, a parallel rapid synthesis of cyclic depsipeptide occurs concomitantly. Their simultaneous occurrence may indicate a common control.

**Introduction**

The red pigment of *Serratia marcescens*, prodigiosin, has strong antibiotic properties but its function *in vivo* is obscure. It may be a secondary metabolite, but if the pigment were of no use to the organism perhaps the ability to synthesize it would have been lost (see Williams & Hearn, 1967). Its function might become clearer by knowing its location in the bacterium, but apart from the report that prodigiosin is in the bacterial envelope with some indications that it may be in the plasma membrane (Purkayastha & Williams, 1960) this is unknown. *Serratia marcescens* also contains large quantities of lipid-bound serine either in the form of serratamic acid (I) (Cartwright, 1955), or its dimer, serratamolide (II) (Wasserman, Keggi & McKeon, 1962).

![Chemical structures](image)

Free serratamic acid is an artifact of alkaline extractions and all the naturally occurring lipid-bound serine is in the cyclic depsipeptide dimer, serratamolide, or its analogues (Bermingham, Deol & Still, 1970a).

We have investigated the relationship between cyclic depsipeptide content and prodigiosin...
synthesis in *Serratia marcescens* since any proven connexion would help to elucidate the function of prodigiosin or cyclic depsipeptide, neither of which is clear at present. The localization of cyclic depsipeptides within the bacterium was also attempted for comparison with the known site of localization of prodigiosin.

**METHODS**

*Cultivation of bacteria.* The following strains of *Serratia marcescens* were used: red-pigmented NCTC 1377 and ATCC 8195; non-pigmented NCTC 4612 and a mutant isolated after u.v. irradiation by ourselves (mutant 214); and for the syntrophic pigmentation studies mutants 9-3-3 and WF were kindly supplied by Professor R. P. Williams, Baylor College of Medicine, Houston, Texas. Cultures were grown in 1 l. conical flasks on a rotary shaker at 30° (unless otherwise stated). Large-scale cultures were grown in 12 l. carboys with continuous aeration at a rate of 4 l./min. Except when stated otherwise, bacteria were grown in the chemically defined medium of Bunting (1946). Mutants 9-3-3 and WF were cultivated on a peptone glycerol medium in the absence of phosphate salts (Jackson, Bunting & Morrison, 1963). Bacteria were harvested by centrifuging at 20,000 *g*, washed with distilled water and freeze-dried.

*Analytical methods.* Cyclic depsipeptides and other lipids were extracted and purified as previously described (Bermingham *et al.* 1970a). Free amino N was assayed by the colorimetric method of Rosen (1957). The cyclic depsipeptide content was determined by measurement of the lipid-bound serine released on 10 m-HCl hydrolysis of the acetone extracts of freeze-dried bacteria (Bermingham, Deol & Still, 1970b). Prodigiosin was assayed visually or by the extinction of a bacterial suspension at 537 nm. according to the method of Williams & Gott (1964).

*Localization of cyclic depsipeptide.* Washed bacteria were suspended in 0.1 M-tris buffer at pH 7.5, frozen at −30° and crushed in a Hughes press. The resulting material was homogenized with a Teflon pestle after addition of DNase and M-MgCl₂ (0.5 ml.) and centrifuged for 1 h. at 64,000 *g* at 1°. The resulting pellet was designated as ‘wall+membrane’ and the supernatant as ‘soluble’ fraction. Each fraction was freeze-dried, extracted with acetone and assayed for cyclic depsipeptide.

**RESULTS**

The production of pigment in *Serratia marcescens* strain NIMA commences only when the exponential phase of growth has ended and continues after the viable count and rate of protein synthesis have declined (Gott & Williams, 1960). A similar pattern of prodigiosin formation was shown by strain NCTC 1377 (Fig. 1) and the cyclic depsipeptide content, which was measured concurrently, increased almost linearly from inoculation to late stationary phase of growth. (In a separate and longer experiment the cyclic depsipeptide content reached a maximum in 48 h. and thereafter remained constant for up to 5 days.)

*Environmental factors.* All strains of *Serratia marcescens* lose their capacity to synthesize prodigiosin when grown above 37°: maximum pigmentation usually occurs between 20° and 30° (Gott & Williams, 1960). Pigmented and non-pigmented strains, including the non-pigmented mutant, were grown at 20°, 30° and 40°, and their cyclic depsipeptide content estimated and pigment production noted simultaneously (Table 1). In pigmented strains the conditions favouring highest prodigiosin content (30°) coincide with those giving rise to maximum cyclic depsipeptide biosynthesis. Also, the strongly pigmented strains contain the highest amount of cyclic depsipeptide irrespective of the source of N.
**Prodigiosin and cyclic depsipeptides**

*Cultural conditions.* Marked variations in pigmentation in *Serratia marcescens* occur in response to changing cultural conditions (Williams & Hearn, 1967). Consequently the production of cyclic depsipeptide and prodigiosin was estimated in bacteria grown on different carbon and nitrogen sources, and at different pH values (Table 2). Variations in environmental and cultural conditions which caused increases in the prodigiosin content almost invariably caused parallel increases in the cyclic depsipeptide content.

![Graph](image)

*Fig. 1.* Comparison of the production of cyclic depsipeptide and prodigiosin with growth in *Serratia marcescens* (strain NCTC 1377). Measurements were made on samples (1 l.) withdrawn from an initial 12 l. of medium in a large carboy. An extinction of 1.5 at 620 nm. corresponds to a value of 950 μg. bacterial dry wt/ml. of growth medium. Symbols: ★, growth rate; ▲, cyclic depsipeptide; ■, prodigiosin.

*Induced pigmentation systems.* (a) Temperature induced system: pigmentation was induced in strain NCTC 1377 grown to the stationary phase at 37° by lowering the temperature to 27° and adding fresh Bacto peptone (50 ml. of a 0.6% solution). Samples of bacterial suspension were withdrawn after 10, 13 and 20 h. and prodigiosin and cyclic depsipeptide content estimated. Prodigiosin was seen after 3 h. incubation at 27° and thereafter increased rapidly up to 20 h. when the bacterial suspension had an extinction at 537 nm. of 0.3, which is a value attained by pigmented bacteria grown at 30°. In the same period the cyclic depsipeptide content of the cells increased sixfold while the increase in growth was only 13%. In a control experiment with bacteria maintained at 37°, and therefore with no prodigiosin being synthesized, no increase in the cyclic depsipeptide content occurred over the same period. (b) Syntrophic pigmentation: mutants 9-3-3 and WF normally do not pigment, but if grown together, or adjacent to one another on an agar plate, can form prodigiosin (Santer & Vogel, 1956; Williams & Green, 1956). These mutants were grown so that the air after
Table 1. Comparison of cyclic depsipeptide content of pigmented and non-pigmented strains of Serratia marcescens grown at different temperatures and harvested at onset of stationary growth phase

Medium A is that of Bunting (1946); with medium B the inorganic N of Bunting's medium was replaced by an equivalent amount of N supplied as casein hydrolysate.

<table>
<thead>
<tr>
<th>Temperature and medium</th>
<th>NCTC 1377</th>
<th>ATCC 8195</th>
<th>NCTC 4612</th>
<th>Mutant 214</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclic depsipeptide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(wt) Cyclic depsipeptide (wt) Pigment* Cyclic depsipeptide (wt) Pigment Cyclic depsipeptide (wt) Pigment Cyclic depsipeptide (wt) Pigment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20° Medium A 0.33</td>
<td>+</td>
<td>0.18</td>
<td>++</td>
<td>0.04</td>
</tr>
<tr>
<td>30° Medium A 0.5</td>
<td>++</td>
<td>1.0</td>
<td>++++</td>
<td>0.1</td>
</tr>
<tr>
<td>40° Medium A 0.3</td>
<td>++</td>
<td>0.8</td>
<td>++++</td>
<td>0.15</td>
</tr>
<tr>
<td>Medium B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30° Medium B 0.3</td>
<td>+</td>
<td>0.12</td>
<td>no growth</td>
<td>-</td>
</tr>
</tbody>
</table>

* +++ = Strong red; +++ = red; ++ = pink; + = faint pink; - = white.

Table 2. Changes in cyclid depsipeptide content of Serratia marcescens (strain NCTC 1377) in response to varying cultural conditions

Bacteria were harvested after 30 h. growth.

Variation of Bunting's medium | Prodigiosin | Cyclic depsipeptide (wt) Pigment
(a) Glycerol+citric acid replaced by an equivalent amount of C supplied as
Glycerol  | +++++ |
Glucose   | ++    |
Fructose  | ++    |
Mannitol  | +     |
(b) NH₄Cl replaced by an equivalent amount of N supplied as
Bactopeptone | +++++ |
Proteoseptone | +++++ |
Neopeptone  | +     |
Bactotryptone | +     |
(c) pH changes
6         | +++++ |
7         | +++++ |
8         | +++++ |

Table 3. Localization of cyclic depsipeptide in different subcellular fractions of Serratia marcescens strain NCTC 1377

Bacteria were grown in 12 l. of Bunting's medium in a large carboy and harvested at stationary growth phase.

<table>
<thead>
<tr>
<th></th>
<th>Intact bacteria</th>
<th>Wall + membrane fraction</th>
<th>Soluble fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry wt recovered (g.)</td>
<td>.</td>
<td>4.97</td>
<td>5.31</td>
</tr>
<tr>
<td>Cyclic depsipeptide (% dry bacterial wt)</td>
<td>0.94</td>
<td>2.28</td>
<td>0.01</td>
</tr>
<tr>
<td>Pigment</td>
<td>++++</td>
<td>++++</td>
<td>-</td>
</tr>
</tbody>
</table>
passing through the culture of WF was passed through the culture of 9–3–3. Under these conditions 9–3–3 pigmented rapidly and its cyclic depsipeptide content was 8.0% (w/w, freeze-dried bacteria) in contrast to 5% when grown in isolation. The cyclic depsipeptide content of both these mutants is unusually high but was not due to the different growth medium used, as strain NCTC 4612 when grown on this medium had an unaltered cyclic depsipeptide content.

Location of cyclic depsideptide. Purkayastha & Williams (1960) showed that prodigiosin is contained in the bacterial envelope. When fractionation and cyclic depsipeptide assay was carried out on the fractions obtained, a similar location was indicated for cyclic depsipeptide (Table 3). Some translocation of components may have occurred when the bacteria were disrupted but this seems unlikely as almost all the cyclic depsipeptide was recovered in the envelope fraction.

DISCUSSION

Although cyclic depsipeptides are not metabolic precursors of prodigiosin (Bermingham, Deol & Still, 1970b, c), these compounds could be associated in other ways. For example, the cyclic depsipeptide might be physically associated with the pigment in the membrane. Such an association of similar components does occur in Rhodopseudomonas spheroides: when the synthesis of bacteriochlorophyll is induced in this bacterium, a parallel rapid synthesis of an ornithine-containing lipoamino acid takes place. The lipid-bound ornithine probably ‘accommodates’ the pigment within the membrane in some way (Gorchein, 1968). However, as the proportions of prodigiosin and lipid-bound serine, i.e. cyclic depsipeptide, vary widely during growth this type of association might be unlikely. Alternatively, the cyclic depsipeptide might be a ‘by-product’ of prodigiosin synthesis; if serine or β-hydroxydecanoic acid (or a precursor of either) was generated in excess during one of the early reactions leading to prodigiosin, the synthesis of a cyclic depsipeptide molecule could trap these metabolites in a metabolically inert form, i.e. both compounds could be secondary metabolites subject to some common control (see Bu’Lock & Powell, 1965). Again, this explanation seems tenuous since the presence of lipid-bound serine has been demonstrated when prodigiosin is absent.

The observed relationship between these two components of Serratia marcescens may simply be a casual one for which the common control mechanism could be the concentration of some simple metabolic intermediate. No function has yet been discovered for either compound, but if they have a related function it might be necessary for both to be present simultaneously for a particular process to occur.

REFERENCES


